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ÚSTAV BIOMEDICÍNSKÉHO INŽENÝRSTVÍ

ANALYSIS OF RELAXATION TIMES OF POLARIZING AGENTS USING RAPID SCAN-EPR

ANALÝZA RELAXAČNÍCH ČASŮ POLARIZAČNÍCH ČINIDEL ZA POUŽITÍ RAPID SCAN-EPR

SEMESTRAL THESIS
SEMESTRÁLNÍ PRÁCE

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VEDOUCÍ PRÁCE

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BRNO **FACULTY OF ELECTRICAL**
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OF TECHNOLOGY **AND COMMUNICATION**

Semestral Thesis

Bachelor's study program **Biomedical Technology and Bioinformatics**

Department of Biomedical Engineering

Student:	Štěpán Zelníček	*ID:*	247326
Year of study:	3	*Academic year:*	2024/25

TITLE OF THESIS:

Analysis of Relaxation Times of Polarizing Agents using Rapid Scan-EPR

INSTRUCTION:

1) Conduct a literature search on the use of electron paramagnetic resonance (EPR) to measure the relaxation times of polarizing agents and their application in dynamic nuclear polarization (DNP).

2) Propose a methodology for detailed measurements of the relaxation times of polarizing agents using Rapid Scan-EPR.

3) Create a database of the relaxation times of various polarizing agents. This database will include not only the relaxation times but also the chemical and physical properties of the radicals obtained through a literature search.

4) Carry out pilot measurements of relaxation times using Rapid Scan-EPR and perform a basic analysis of the data obtained.

5) Propose the structure and implementation of a database of relaxation times and related properties of polarizing agents.

6) Discuss the obtained results and evaluate the effectiveness of the methodology and the potential use of the database in scientific research and industrial applications.

To fulfill the requirements of the semester project, it is necessary to complete at least points 1) to 3).

RECOMMENDED LITERATURE:

[1] Neugebauer, P., Krummenacker, J., Denysenkov, V., Parigi, G., Luchinat, C., & Prisner, T. (2013). Liquid state DNP of water at 9.2 T: an experimental access to saturation. Phys. Chem. Chem. Phys., 15, 6049-6056.

[2] Neugebauer, P., Krummenacker, J., Denysenkov, V., Helmling, C., Luchinat, C., Parigi, G., & Prisner, T. (2014). High-field liquid state NMR hyperpolarization: a combined DNP/NMRD approach. Phys. Chem. Chem. Phys., 16, 18781-18787.

[3] Laguta, O., Sojka, A., Marko, A., Neugebauer, P.; Rapid scan ESR: A versatile tool for the spin relaxation studies at (sub)THz frequencies. Appl. Phys. Lett. 21 March 2022; 120 (12): 120502. https://doi.org/10.1063/5.0083010

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doc. Ing. Jana Kolářová, Ph.D.
Chair of study program board

Abstract

An abstract in the English language (replace this section with your own text).

Generally, an abstract contains reduced characteristics of the document; thus, it does not comprise information relating to the authors, supplementary data, or individual evaluation of the thesis (namely, sentences such as "*In this thesis, I describe in an effective way a system that I consider interesting*" should be shortened and made neutral to read "*The thesis describes a system*". The actual text is expected to be concise, clear, accurate, objective, easy to read, and formulated naturally in written sentences. Although the author may use phrases or passages from his or her own term papers and/or theses, these together must form a new whole. A standard abstract is 5 to 10 lines long, with 50 to 100 words not exceeding 1,000 characters.

Keywords

Relevant keywords in the English language (replace this section with your own text). This part of the thesis specifies the most significant words or phrases that, within the limits of the natural language, allow the thesis to be suitably classified, searched, and identified. The keywords should match relevant entries in the thesaurus of the given discipline and are expected to characterize the problem discussed in the author's term paper(s) and/or theses. The keywords should be ordered logically, in a hierarchical manner or according to the contents of the thesis. In terms of quantity, 5 - 8 keywords are considered standard.

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Ekvivalentní text abstraktu v českém jazyce (odstavec nahraďte svým textem).

Klíčová slova

Ekvivalentní klíčová slova v českém jazyce (odstavec nahraďte svým textem).

Rozšířený abstrakt

Extended abstracts must contain all relevant aspects and information of the thesis.

Bibliographic citation

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Author's Declaration

Author:	Štěpán Zelníček
Author's ID:	247326
Paper type:	Semestral Thesis
Academic year:	2024 / 25
Topic:	Analysis of Relaxation Times of Polarizing Agents using Rapid Scan-EPR.

I declare that I have written this paper independently, under the guidance of the advisor and using exclusively the technical references and other sources of information cited in the project and listed in the comprehensive bibliography at the end of the project.

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You can formulate your own acknowledgment to those who helped you with your work (supervisor, consultant, colleague, ...). Insert the original signed acknowledgment into the printed version of the final thesis; an unsigned acknowledgment is included in the electronic version.

Example:
I would like to thank my supervisor ………. for his invaluable advice and support.

Brno, December 18, 2021   --
                                                                          Author's signature

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1. GENERAL EPR TECHNOLOGY

To know what are we gonna talk about.

1.1 General spectroscopy

To begin to have an idea of what was sought in this work, the reader should know a little of what spectroscopy involves. Spectroscopy is the study of electromagnetic radiation concerning its production [luciferaza] and interaction with matter for quantitative and qualitative analysis. Electromagnetic radiation can be considered a disturbance travelling through space, made up of electric and magnetic components. Unlike the magnetic field associated with a common magnet or the Earth, the magnetic field linked to electromagnetic radiation continually changes both in direction and strength. Similarly, the electric field component of the radiation also continually undergoes a change in direction in the same manner (see Figure 1.1).

Figure	1.1	An electromagnetic wave in which an electric (E) and magnetic (B) field exists at right angles to one another. The oscillation of the electric component generates the magnetic field; this in turn generates a new electric component which allows the wave to propagate through space.

There are several important fundamental properties of electromagnetic radiation. The periodicity of the disturbance, or the distance between consecutive troughs or peaks, is called the wavelength of the radiation. While frequency denotes the number of complete cycles undergone by the electromagnetic field in a unit of time. The whole spectra of

electromagnetic waves are shown on the Figure 1.2.

γ-ray	X-ray	UV		IR	MW		RF

Figure 1.2   Regions of the electromagnetic spectrum and associated energies, frequencies and wavelengths with set regions of EPR and NMR, where E / kJ mol-1 is the energy per mol, the ν is the frequency in Hertz (Hz) and λ is the wavelength in meters (m).

Radiation[1] and its interaction with significant yield requires that the frequency of electromagnetic oscillations generated by radiation must exactly match the oscillations of the electromagnetic moments of the material. This resonance ensures that energy is efficiently transferred; hence, it allows for a stronger interaction between the radiation and matter encountering it. Without this matching of frequencies, the potential for meaningful interaction is greatly diminished. The situation is rather like that of pushing a person on the swing. That is to say, if a person sitting on a swing completes a cycle every second, then pushing at all other frequencies will make the transfer of energy inefficient. Energy is transferred most efficiently when pushes are matched with one full cycle. During the process of interaction between radiation and matter, several events may occur. From those, the most important to spectroscopy will be absorption emission, and scattering.

---

[1] Radiation = electromagnetic wave propagation.

2. FUNDAMENTALS FOR MAGNETIC RESONANCES

This chapter is dedicated to understanding the EPR theory and the principles behind it in order to understand the following measurements.

2.1 The Spin
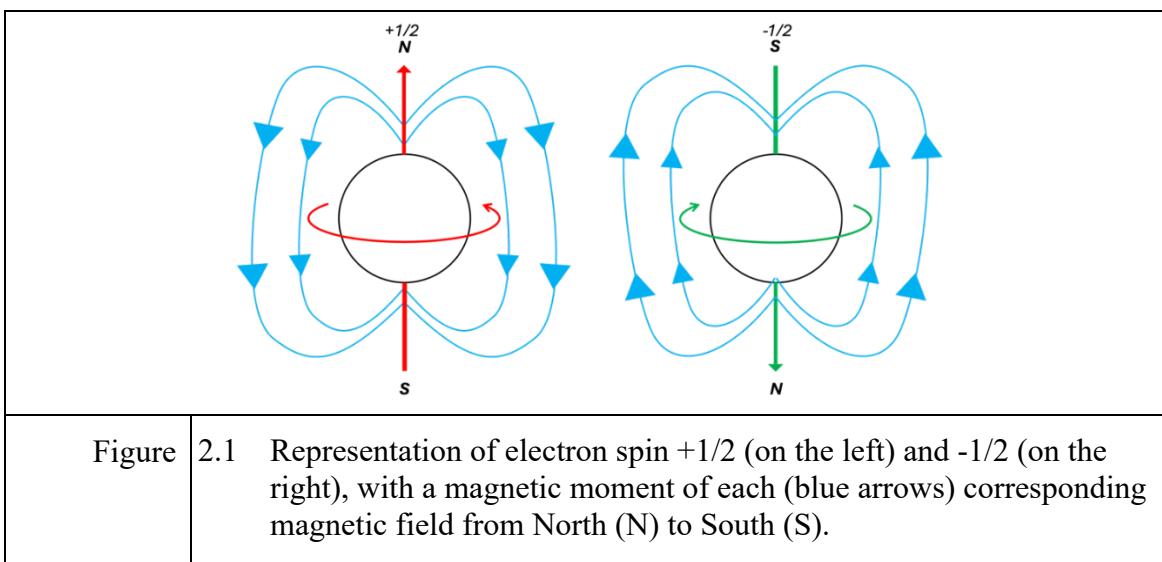
Even in the 21st century it is hard to grasp and understand the concept of spin, but the spin can be almost described as a small magnet (see Figure 2.1) with its own magnetic field and direction, therefore it would be described in this sense later in this section.

2.1.1 The electron spin

The electron is a negatively charged particle which possesses an intrinsic property called spin. Spin is a quantum feature which describes its inner moment of momentum, but it is not rotation in a classical meaning[2] like for example rotation of a ball around its own axis. Spin has some important characteristics:

1. Quantum number of spin
   a. Spin is quantized, which means it can only swell certain values. Each particle has its specific value, which could be as a whole number (0, 1, 2, ...) or a half-number (1/2, 3/2…). Electron has a spin number of 1/2.

2. The direction of the spin
   a. For the particles with spin 1/2, like electrons, the direction of their spins is labelled as "up" (+1/2) and "down" (-1/2), which corresponds with two different orientations towards the appointed direction (for example towards an external magnetic field (see also section Ground and excited state [libretext, bolton]).

3. Spin magnetic moment
   a. Because spin relates to the momentum, each electron (or any other particle with spin) also has a magnetic moment. This means that electron function also functions as a small magnet which can be oriented in a magnetic field (see section Zeeman effect). This is crucial for technologies and methods such as NMR or EPR.

---

[2] There are many theories and looks on what the spin actually is such as the Pauli's "classically non-describable two-valuedness" which is more closely to how the spin is described in this work but still considering the spin as an abstract property [link]. Another theory is the Dirac's relativistic electron theory which requires the Dirac relativistic wave equation [link]. This chapter about spin is simplified version and analogy to small magnet, but it more abstract then that.

Figure	2.1	Representation of electron spin +1/2 (on the left) and -1/2 (on the right), with a magnetic moment of each (blue arrows) corresponding magnetic field from North (N) to South (S).

2.1.2 The nuclear spin

Not only the electron but also the nucleus has a spin that is characterized by I and it can vary between 1/2 to even 5/2 or more (can also be whole numbers). All the previous chapters that discussed electron spin behaviour in the presence of an external magnetic field, also apply to the nuclei but with some minor changes. The nuclei is able to have spin due to the nucleons that it's made of, which are protons and neutrons. Both have intrinsic angular momentum. So, the overall spin of nuclei I comes from the combined spins of these nucleons. For an even number of protons and neutrons, the total spin would be $I = 0$ (such as ^{12}C or ^{16}O). For an odd number of protons or electrons, the nucleus has a half-integer spin such as ^{1}H with $I = 1/2$ or ^{13}C with $I = 1/2$. For an odd number of protons and neutrons, it can have an integer spin (^{14}N with $I = 1$) [nmr nomenclature].

2.1.3 Larmour frequency

2.1.4 paramagnetismus,

2.1.5 ferro (how there are alling) {magnetization}

2.2 Ground and excited state [libretext, bolton]
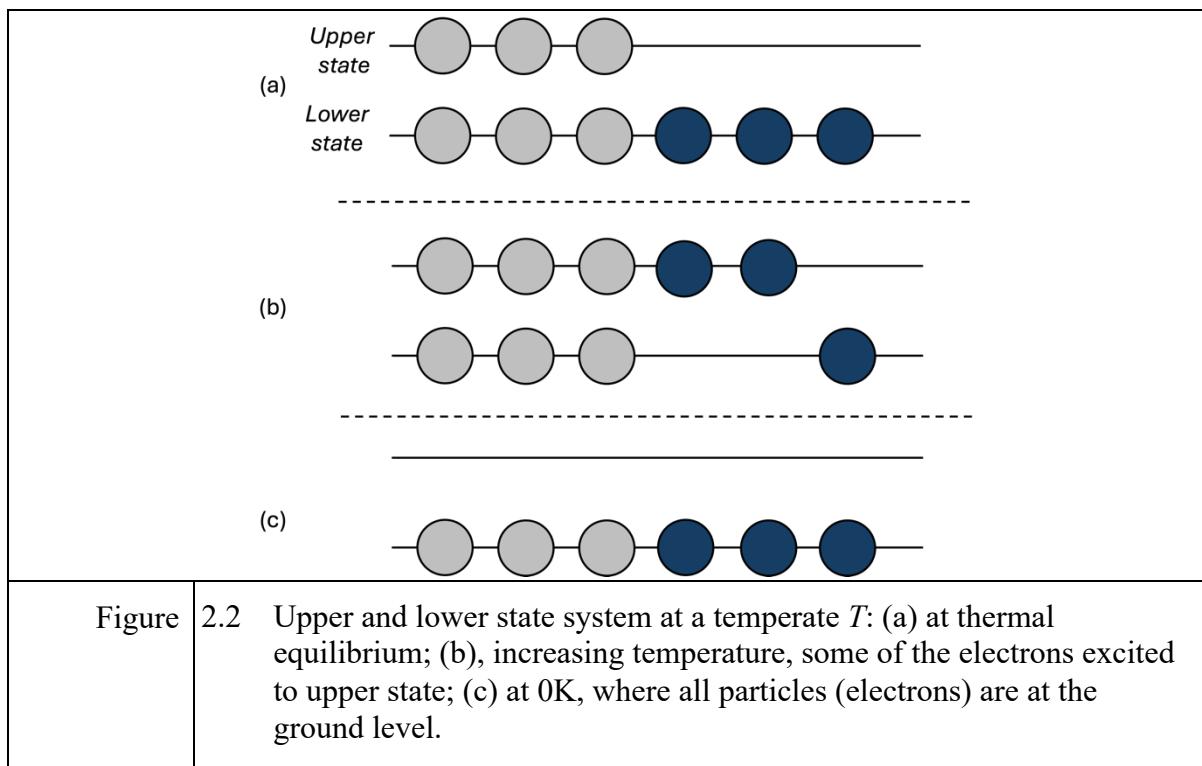
When the system reaches a point of thermal equilibrium, the electrons' spins specifically, can be in either of two states: +1/2 and –1/2 (for a free particle (electron)). These two states are referred to as the *ground state* and *excited state*, with the ground state being the -1/2 spin and the excited state the +1/2 spin. For a temperature above 0 K, there will inevitably be a distribution in the population of these states. This population is explained by a Boltzmann's population theory or also referred as distribution theory (eq. 2.1). The

theory states that a system occupies a specific state at the condition of energy of such states and the temperature of the system. This phenomenon is expressed by the equation below:

$$\frac{N_{upper}}{N_{lower}} = e^{-\frac{\Delta E}{kT}}$$ (eq. 2.1)

Where N_{upper} is the count of the particles in the upper (excited) state, N_{lower} is the count of particles in the lower (ground) state, ΔE is the energy difference between the ground and excited state, k is the Boltzmann's constant (1.380649×10^{-23} m² kg s⁻² K⁻¹), and T is the absolute temperature of the system.

Since nature always favours states with lesser energy, there is a tendency for particles to be in the ground state rather than in the excited state. Theoretically, then, at 0 K, the temperature wherein the particles do not have any internal motion from the temperature of the system, the particles would be wholly in the lowest energy state ground state (see Figure 2.2). Here to explain more temperature dependency.

Figure 2.2 Upper and lower state system at a temperate T: (a) at thermal equilibrium; (b), increasing temperature, some of the electrons excited to upper state; (c) at 0K, where all particles (electrons) are at the ground level.

2.3 Absorption

By recalling that absorption is the one of the spectroscopic methods by which we are able to perform analysis on the samples that we examine. Now consider a Boltzmann population of +1/2 and -1/2 spins at energies: the distribution across this population goes by the name Boltzmann factor, while the energy states over which it is distributed goes under the name Boltzmann population. We can consider analogies between electrons and nuclei. So under similar conditions such as temperature and pressure, the difference in population from the ground state (-1/2 spin) to excited state (+1/2 spin) is rather low compared to that of electrons. [bolton] When energy is applied onto this system, a certain number of particles jump into excited state and when energy is removed, they "fall" back to their original equilibrium state. That is absorption process. The process is greatly influenced by temperature. [epr primer],

At high temperatures, the population difference becomes smaller-that is, the population of the ground state and excited state approaches each other-leading to a smaller transition from the lower to the upper state, hence less intensity of the signal. For lower temperatures, energy level difference becomes larger, and the population of the ground state will dominate the upper state. If absolute zero at 0K were attainable, theoretically, all the population would be in the ground state and there would be no population in excited states.
[chechick, a consistent description of epr spectra 1990]

2.4 Zeeman effect

2.4.1 Electron Zeeman effect

The absorption process described earlier was considered only in the absence of an external magnetic field. But what happens to the energy levels when the magnetic field is applied? Firstly, we said that when a system is at thermal equilibrium, its spin is so-called degenerate, meaning there are discrete levels of energy for the alpha and beta electron spin, which has no difference in the population. However, when the external magnetic field is applied, these levels start to separate, and we can now distinguish the difference in the levels. This effect is called Zeeman splitting or Zeeman effect and it was discovered by Pieter Zeeman in 1896, a year before the discovery of the electron, and so this effect was explained after the electron spin was discovered [Relaxtimes]. For this, he was granted a Nobel Prize. What he found out was that when a ray of light is influenced by the magnetic field, its beam splits into separate lines, hence into different energy levels. Similarly, for the magnetic resonance technique in general, the Zeeman splitting is fundamental to comprehend. As the strength of the magnetic field increases, the separation of the different energy levels of electron spins (ground state and excited state)

increases as well (see Figure 2.3). In classical terms, the interaction energy of the electron magnetic moment with B is given by:

$$E = m_s \mu_B B \qquad \text{(eq. 2.2)}$$

Where, E is the Zeeman energy, ms is the spin, μs is Bohr's magneton for electron and B is the strength of the external magnetic field. [epr primer].

The Bohr's magneton is a natural unit in which to measure the magnetic moment of an electron, denoted by:
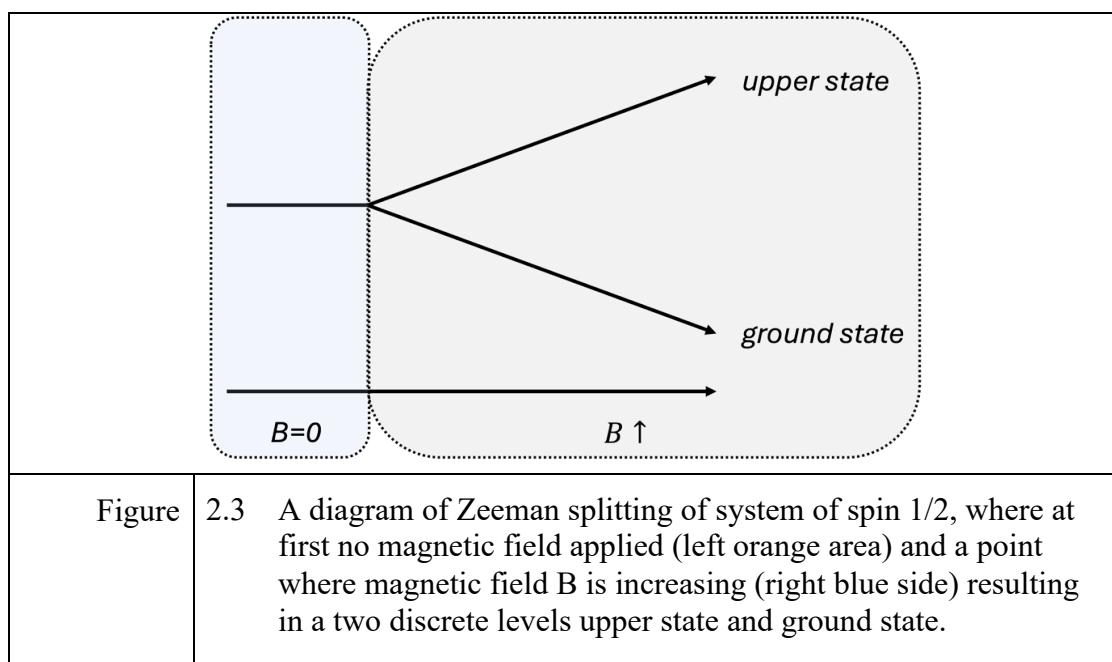
$$\mu_B = \frac{e\hbar}{2m_e} = 9.274 \cdot 10^{-24} J\ T^{-1} \qquad \text{(eq. 2.3)}$$

Where e the elementary charge of an electron, $\hbar$ is reduced Plank's constant ($\hbar = \frac{h}{2\pi}$) and m_e is the mass of the electron.

So far, we can say that the energy E is directly proportional to the magnetic field B. When we rewrite the equation with the magnetic dipole moment, the final equation would be:

$$E = g_e m_s \mu_B B \qquad \text{(eq. 2.4)}$$

Where only new term is the g_e which is the g factor of the **free electron** of value 2.002316 (described in section factor).

| Figure | 2.3 | A diagram of Zeeman splitting of system of spin 1/2, where at first no magnetic field applied (left orange area) and a point where magnetic field B is increasing (right blue side) resulting in a two discrete levels upper state and ground state. |

2.4.2 Nuclear Zeeman effect

It was demonstrated in the above sections that energy of both electron spin states depends on the orientation of magnetic dipole moment in the applied field, B, such that two electron Zeeman levels (ground and excited states). However, as long as a non-zero nuclear spin quantum number I, there will also be associated magnetic moment, μ_I, and hence the Zeeman splitting takes place in the magnetic field. The nuclear magnetic moment, μ_I, can be described as:

$$\mu_I = g_N \mu_N I \qquad \text{(eq. 2.5)}$$

Where g_N is the effective nuclear g factor (which can have a positive or negative value see Table 2.1), μ_N is the nuclear magneton, which is defined by the equation below:

$$\mu_N = \frac{e\hbar}{2m_p} = 5.0508 \cdot 10^{-27} J\, T^{-1} \qquad \text{(eq. 2.6)}$$

Where the only undefined variable is the m_p, that is the mass of a proton.

I	Nuclide
0	^{12}C, ^{16}O
1/2	^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{29}SI, ^{31}P
1	^{2}H, ^{14}N
3/2	^{11}B, $^{35,37}Cl$, $^{64,65}CU$
5/2	^{17}O, ^{55}Mn
7/2	^{51}V

Table 2.1   Description of nuclear spin of the common nuclides.

Similar to the spin angular momentum of electron, the magnitude of the nuclear spin moments is also calculated by the equation:

$$|I| = \sqrt{I(I+1)} \qquad \text{(eq. 2.7)}$$

So as a result, the vector I can assume $2I+1$ discrete orientations which are given by the magnetic quantum number m_I:
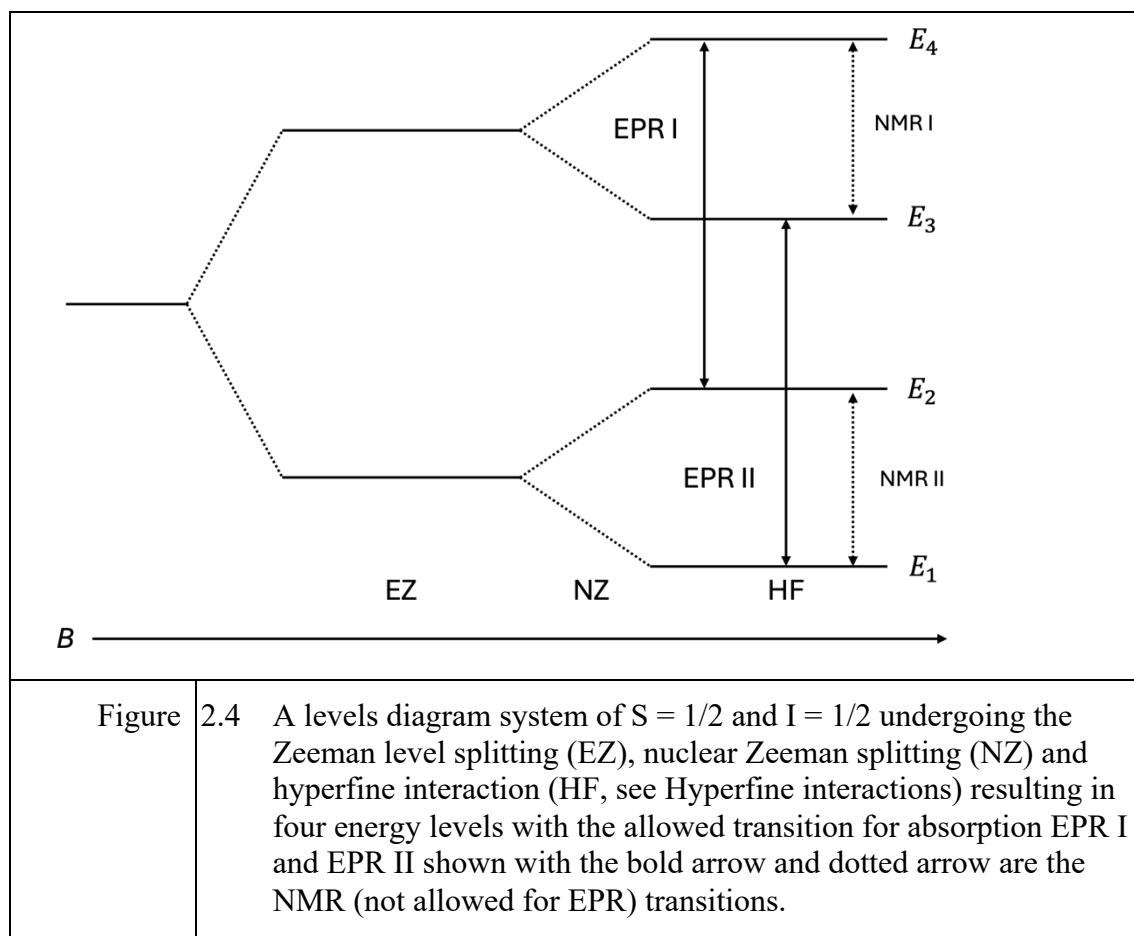
$$m_I = -I, -I+1 \ldots I-1, I \qquad \text{(eq. 2.8)}$$

Hence, the same way as for the electron we can calculate the energy when external energy

is applied:

$$E = g_N m_I \mu_N B \qquad \text{(eq. 2.9)}$$

The additional energy that comes from the nuclear Zeeman splitting, is splitting the already split energies of the electron Zeeman effect as illustrated in Figure 2.4 for two spin systems ($S = I = 1/2$). Each of the non-degenerate electron Zeeman levels is split into $2I+1$ nuclear Zeeman levels. So for the system of $I = 1/2$, there will be a total of 4 discrete levels (four energy levels labelled as E_1 to E_4). **BOLTON, CHECHICK**

Figure 2.4   A levels diagram system of S = 1/2 and I = 1/2 undergoing the Zeeman level splitting (EZ), nuclear Zeeman splitting (NZ) and hyperfine interaction (HF, see Hyperfine interactions) resulting in four energy levels with the allowed transition for absorption EPR I and EPR II shown with the bold arrow and dotted arrow are the NMR (not allowed for EPR) transitions.

2.5 Resonance condition [consistent description of epr spectra]

This brings us to the resonance condition. This condition is used in EPR spectroscopy, NMR spectroscopy, and others, where the Zeeman splitting and absorption play a crucial role. We have discussed what is absorption and what are the needs for it to occur. Another important role in order to observe the peak in EPR spectra is the Zeeman splitting (Zeeman effect) which separates the energies of a system to discrete levels (ground and excited) (see Figure 2.5). When absorption and Zeeman splitting come hand in hand, we can define the resonance condition, in which we can achieve the EPR spectra peak. First, we determine the energy difference:

$$\Delta E = g_e \mu_B B \qquad \text{(eq. 2.10)}$$

For the absorption to happen, there must be given a specific energy of a photon to the system which flips the spins from the ground state to the excited state, this energy is equal to:

$$E = h\nu \qquad \text{(eq. 2.11)}$$
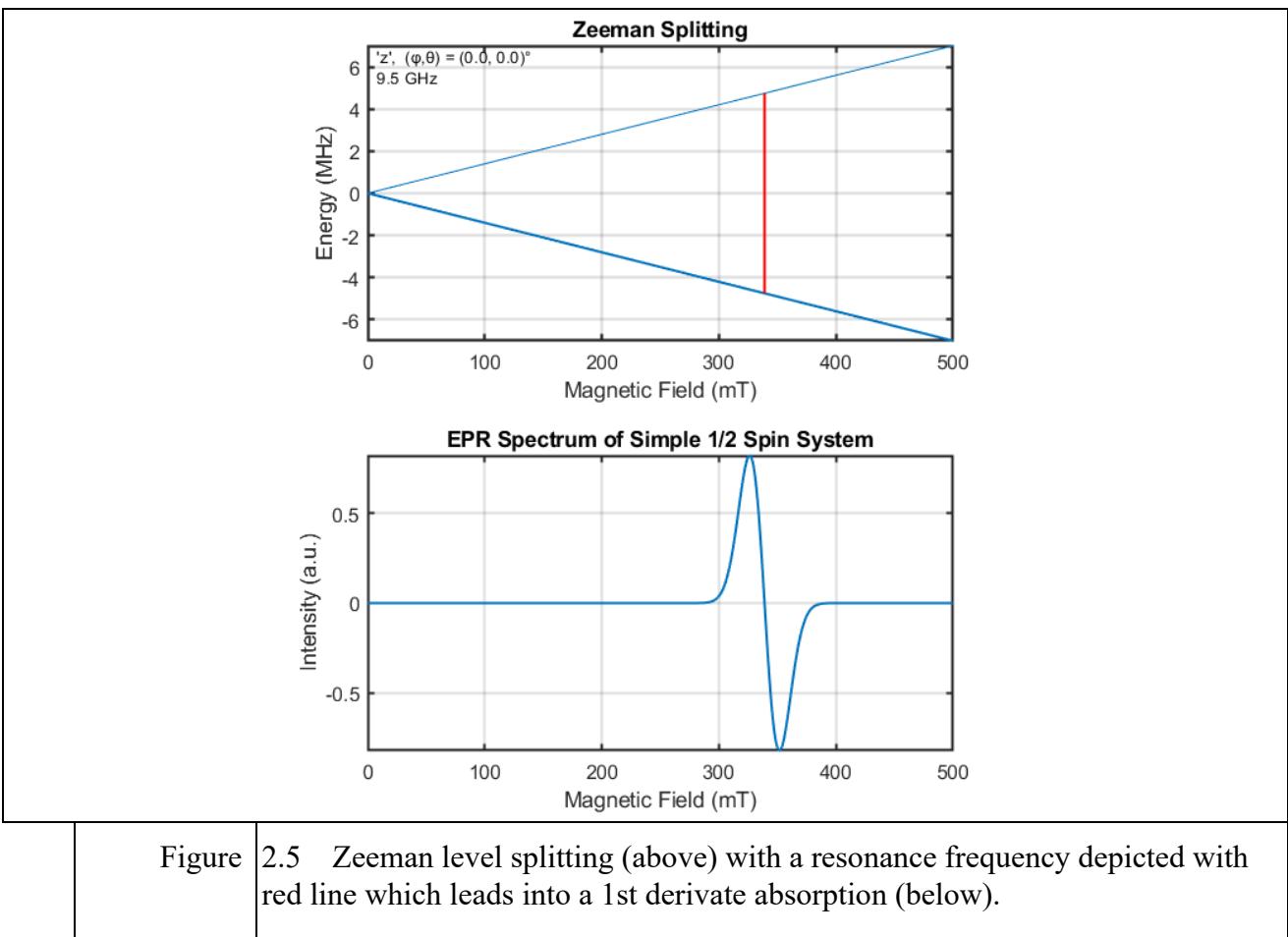
Where h is the Plank's constant, and ν is the frequency of radiation used. Combine this equation and we get the resonance condition:

$$\Delta E = g_e \mu_B B = h\nu \qquad \text{(eq. 2.12)}$$

Indeterminately, the resonance condition is linear[3] and is dependent on the magnetic field B and the frequency used to irradiate the system (see Figure 2.5).

---

[3] It is a fact, that at the "lower" magnetic field, less then 10 T, the Zeeman splitting is linear, but when we go beyond this point, the filed becomes non-linear. Between 10 T and 20 T, the field is mixed (linear and non-linear), but above 20 T, this non-linearity needs to be considered and count on. Though is not a practical problem due to do fact, that only few lavatories uses this high field. [zeeman level from 1933]

Figure	2.5 Zeeman level splitting (above) with a resonance frequency depicted with red line which leads into a 1st derivate absorption (below).

2.6 G-factor and effects of symmetry

2.6.1 G factor

As mentioned in the Zeeman effect and the Resonance condition [consistent description of epr spectra] chapters, the energy level depends on the magnetic field, Bohr's magneton and a *g* factor (see). The *g* factor, or the Landé *g* factor, is a dimensionless quantity that represents a constant of proportionality between the magnetic field and the energy difference between the energy levels of the system [g_factor]. The *g* value acts as a unique fingerprint to understand what is being measured [epr primer]. The calculation of the resonance condition can acquire the *g* value. The *g* factor is independent of the microwave radiation, so it is mainly dependent on the magnetic field. The *g* factor of free electron is around 2.002319. Most organic radicals (with which are later the work focusing on) have *g* values between 1.99 and 2.01, while transition metal species have a wider range of *g* values (from around 1.4 to about 4) [g factor metals] . For lighter atomic masses like those in organic radicals, the *g* value is usually close to that of free spin.

CHECHICK, THIS BOOK

2.6.2   Isotropy and Anisotropy

To mentioned, it is important in many EPR methods (see Types of EPR) to know, whether the sample is so-called isotropic or anisotropic. Isotropy means homogeneity in all directions, and on the other hand, anisotropy is the property of being directionally dependent [the paper above]. This results in changing the g factor in the magnetic field. Let's say we isotropic system of free radicals (therefore unpaired electron) in solution. The g factor would have the same value no matter the orientation of the magnetic field, it acts as a scalar value, which significantly helps to interpret the spectra. To contrast that, imagine the anisotropic system, the g factor is changing with the orientation of the magnetic field. The g factor has different values in different axes and therefore it cannot be described only by one number by three numbers in a matrix g_x, g_y, g_z. In practice, there are more anisotropic systems rather than isotropic systems.

There are specific terms for different anisotropies. Axial and rhombic.[paper]

Axial happens when:

$$g_x = g_y \neq g_z \quad\quad\quad\quad\text{(eq. 2.13)}$$

And rhombic:

$$g_x \neq g_y \neq g_z \quad\quad\quad\quad\text{(eq. 2.14)}$$

Even though the anisotropy might be harder to interpret in the final spectra, it helps us to determine the surroundings of the paramagnetic centre which we are measuring. This concept is also a variable in a later chapter Spin relaxation mechanisms and RAPID SCAN – EPR.

Ways to get rid of anisotropy as well?
EQ 2.12 **CHECHICK**
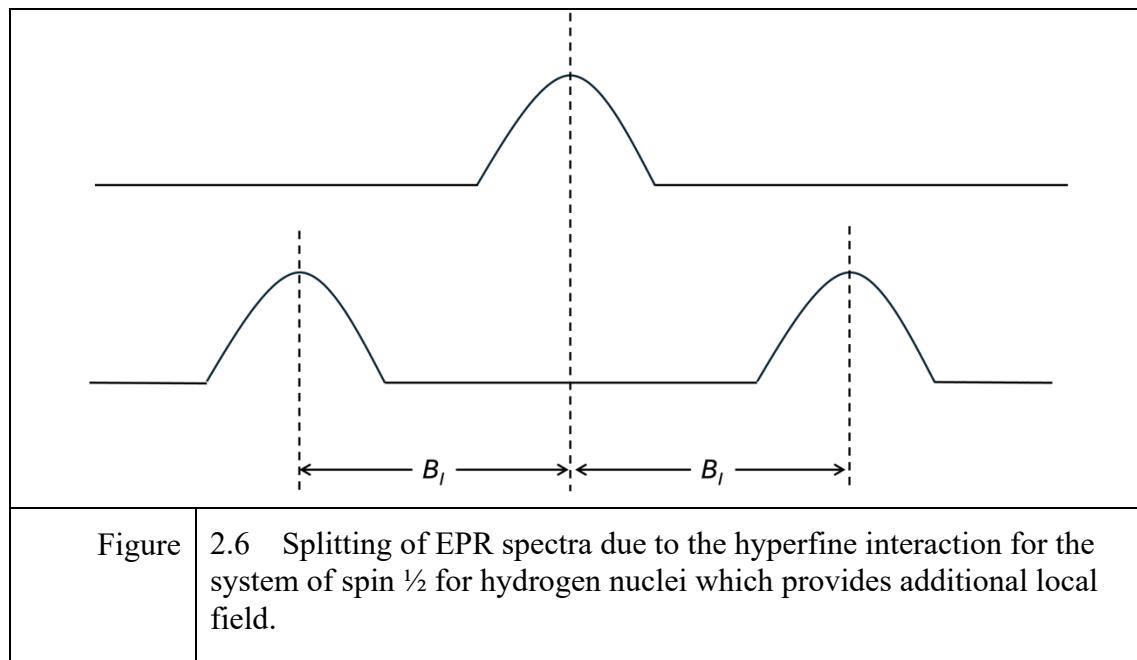
2.7   Hyperfine interactions

Measuring only the g factor gives very little information about our sample molecular structure. The most important advantage in EPR is the sensitivity of the unpaired electron to its local environment: it follows that any interaction with magnetic dipoles of nearby nuclei and vice versa, generates a magnetic field which interacts with the electron and hence becomes apparent on the final EPR spectrum. The interaction of electrons with nuclei is called hyperfine interaction. This interaction will tell us a great deal about our sample, for example, the type and number of atoms making up a radical or complex and the distance of all these atoms from an unpaired electron.

The nuclei acts like a bar magnet, which either opposes or adds to the magnetic field from the laboratory magnet, depending certainly on the alignment of the moment of the nucleus. Hence, when B_I (magnetic field of the nucleus) adds to the magnetic field, we need less magnetic strength from the laboratory system, and when B_I opposes, we need more magnetic field from the laboratory magnet. [**other**]

If we take for a demonstration a spin 1/2 nucleus, such as the hydrogen nucleus, we observe that the absorption EPR signal splits into two signals, each B_I away from the original resonance centre (see Figure 2.6).

Figure 2.6 Splitting of EPR spectra due to the hyperfine interaction for the system of spin ½ for hydrogen nuclei which provides additional local field.

The solution of Figure 2.6 (see Nuclear Zeeman effect) for the corresponding energies of the two-spin systems ($m_S = \pm\frac{1}{2}, m_I = \pm\frac{1}{2}$) are given by:

$$
\begin{aligned}
E_1 &= -\frac{1}{2}g_e\mu_B B - \frac{1}{2}g_N\mu_N B - \frac{1}{4}a \\
E_2 &= -\frac{1}{2}g_e\mu_B B + \frac{1}{2}g_N\mu_N B + \frac{1}{4}a \\
E_3 &= +\frac{1}{2}g_e\mu_B B - \frac{1}{2}g_N\mu_N B + \frac{1}{4}a \\
E_4 &= +\frac{1}{2}g_e\mu_B B + \frac{1}{2}g_N\mu_N B - \frac{1}{4}a
\end{aligned}
\qquad \text{(eq. 2.15)}
$$

Where the first element is the electron Zeeman splitting, the second term is the nuclear Zeeman splitting and a is the hyperfine interaction. The hyperfine a is only 1/4 due to the even distribution between all levels. We can notice, with the help of Figure 2.6, that only transitions that are allowed are from the lower to the upper state, so from the lower

electron Zeeman term $(-\frac{1}{2}g_e\mu_B B)$ to the upper $(+\frac{1}{2}g_e\mu_B B)$ and also the opposite sign of a and the nuclear state must remain the same. Therefore, the only allowed transitions are EPR I (E_2 to E_4) and EPR II (E_1 to E_3). Other transitions are forbidden. **CHECHIC**

2.8 Spin relaxation mechanisms

One of the main topics, this work focuses on, are the relaxation times. The relaxation times describe how fast the system of electrons (for EPR) returns to its original state after being excited. The study of relaxation times is crucial for the DNP's (see DNP) later-explained optimized choice of polarizing agents (see Polarizing agents). The relaxation times are being studied due to their interaction with electrons between them and due to their interaction with the surrounding lattice (the environment that the system is in). This interaction results in the relaxation mechanisms that act to restore the system to equilibrium. One of the most valuable tools for discussion of relaxation times is provided by the Bloch model. BOLTON

2.8.1 Bloch Model

The Bloch model is a theoretical way of explaining the dynamics of magnetization. It was brought by Felix Bloch in 1946, and it helps to describe how magnetization is behaving under the influence of external magnetic field, including relaxation. First will be presented the whole concept of the Bloch model, and then explained both of specific relation times and their meanings.

First thing first, let $\boldsymbol{M}$ be the bulk magnetization with components of M_x, M_y and M_z. Using the convention of aligning $\boldsymbol{B}$ along the z-direction, if the system remains in thermal equilibrium with the lattice, the resulting magnetization will be directed along the z axis due to the excess of spins in the lower energy of ground spin state. If the relative population of the upper and lower spin states are changed by the absorption of microwave energy, the bulk magnetization is forced out of equilibrium. The time evolution of the total spin magnetization vector is described in the presence of static magnetic fields as a function of the spin-lattice (T_1) and spin-spin (T_2) interaction (later explained) mechanism. The Bloch equations for the components of the bulk magnetization are:

$$\frac{dM_x}{dt} = \gamma_e B M_y - \frac{M_x}{T_2} \qquad \text{(eq. 2.16)}$$
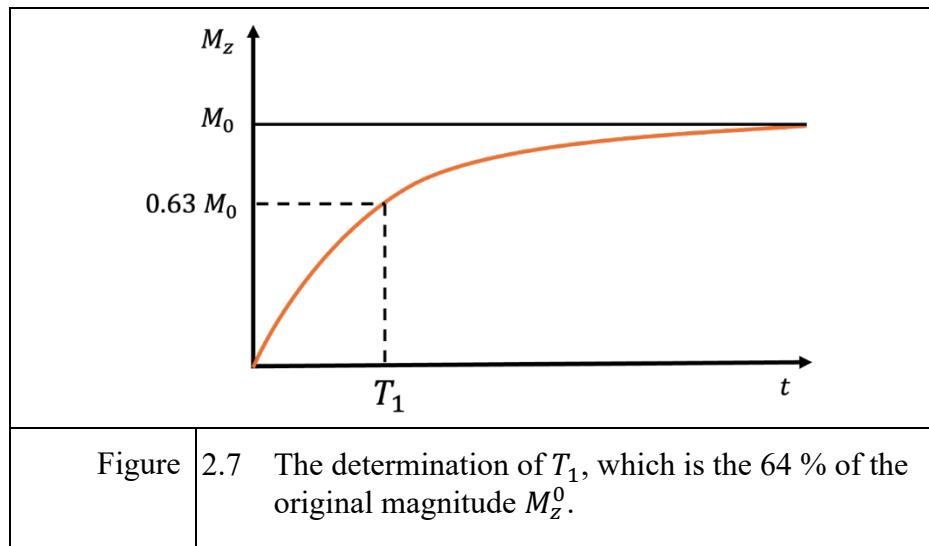
$$\frac{dM_y}{dt} = -\gamma_e B M_x - \frac{M_y}{T_2} \qquad \text{(eq. 2.17)}$$

$$\frac{dM_z}{dt} = \frac{M_z^0 - M_z}{T_1} \qquad \text{(eq. 2.18)}$$

Where M_x, M_y and M_z are the already mentioned components of bulk magnetization, γ_e is the electronic magnetogyric ratio (equal to $g\mu_B / \hbar$), T_2 is the spin-spin interaction, T_1 is the spin-lattice interaction. We can notice from the ((eq. 2.16) and (eq. 2.17) that the time evolution of the M_x and M_y components is characterized by the spin-spin (T_2) relaxation time. The deviation of the z component of the magnetization M_z from equilibrium value M_z^0 is restored through spin-lattice interaction (T_1). CHECHICK

2.8.2 Longitudinal relaxation time T_1
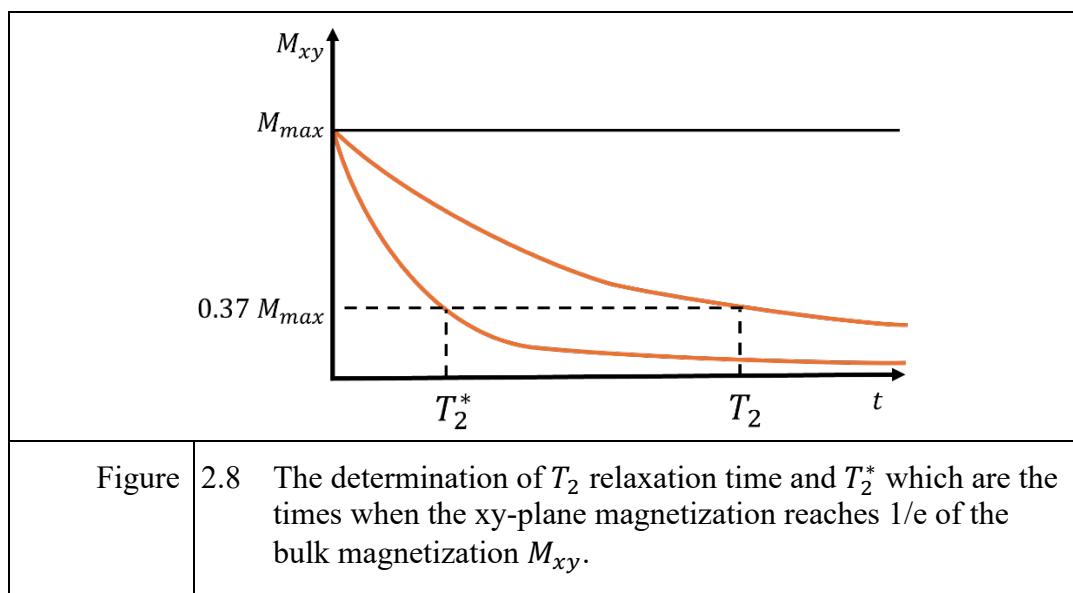
The spin-lattice relaxation relates to the time of how long it takes the system to release the energy to its surroundings (lattice) and to come back to thermodynamic equilibrium. This type of relaxation affects the recovery of magnetization parallel to the main field B_0, which is why it is referred to as longitudinal. Initially, in equilibrium, the net magnetization vector aligns with the magnetic field B_0 along the z axis. When a pulse is introduced, it can flip this magnetization to xy-plane reducing the z component (longitudinal) of the magnetization. This relaxation involves an energy transfer from the spin system to the lattice, which gradually return their lower energy, equilibrium state aligned with B_0 [this book]. If we would want to measure the time of T_1 relaxation, we would record the time for which the z magnetization M_z would recover 63 % of its original magnitude M_z^0 [honza nmr] (see Figure 2.7).

Figure 2.7 The determination of T_1, which is the 64 % of the original magnitude M_z^0.

2.8.3 Transverse relaxation T_2

The spin-spin relaxation on the hand is used to quantify the rate of the decay of the magnetization in the xy-plane, therefore transversal relaxation. It is a specific type of relaxation in which comes the loss of the coherence of each magnetic moment vectors due to their natural process moment of movement (meaning that not all population will have the same relaxation and precession, each particle has slightly different resonance

frequency, so even though they are all alignment either with or against the external magnetic field, there still will be a presses motion on its own) which results in the loss of bulk magnetization for the xy-plane. The measurement of the transverse relaxation would be measured in the same terms as the longitudinal relaxation, meaning, is the is the decline of bulk magnetization of the M_{xy} to 37 % of its maxima (see Figure 2.8). In practice, we usually encounter the relaxation constant T_2^*, which slope is steeper then T_2, which is due to the changes of inhomogeneity of the external magnetic field (which results in even faster loos of coherence). For the later experiments, the resulting relaxation times will be acquired from the equations of the Bloch model (see Modified Bloch equations). [honza nmr].

| Figure	2.8	The determination of T_2 relaxation time and T_2^* which are the times when the xy-plane magnetization reaches 1/e of the bulk magnetization M_{xy}.

[Anisotropy changes t2 paper]

3. TYPES OF EPR

There are many types of electron paramagnetic resonance. All differ and all has advantages and disadvantages. For this work and for understanding the relaxation times, the method of Rapid Scan – EPR has been performed and will be discussed more in detail. It is also notable to mention some common, more used EPRs to understand the concept that has been mentioned earlier. Both of technique, Continuous Wave EPR (CW-EPR) and Pulse EPR can measure the relaxation times, but the Rapid-Scan has some advantages compared to CW and Pulse EPR, but CW- EPR method of acquiring the relaxation time will be used to validate the results from the Rapid Scan EPR.

3.1 CW - EPR and PULSE – EPR

Continuous Wave and Pulse EPR are the most used methods of EPR in practice. CW, as the name hints, is a continuous wave mode, meaning one specific frequency is chosen and the magnetic field is being "swept" (or change). This way, the resonance condition (see (eq. 2.12) is met, via the changing the "gap" energy between the levels. There are also the opposites, where the magnetic field is stable, and the frequency is changed (this is used the NMR imaging in such places as hospitals or etc.). The detection of absorption, not only in CW, is the detection of reflected microwave power from the sample (so the dispersion). [BOLTON, CHECHICK]

PULSE EPR is a technique wherein short, precisely timed microwave pulses are used to manipulate and study the behaviour of unpaired electron spins in a magnetic field (see Figure 3.1). By applying these pulses and observing how the spins respond over time, one measures important properties such as relaxation times (T_1 and T_2), reflecting the interactions of spins with their surroundings and with each other. It gives detailed insight into the dynamics of the electron spin and its surroundings, which is useful for studying properties of molecules and materials. [This chapter]
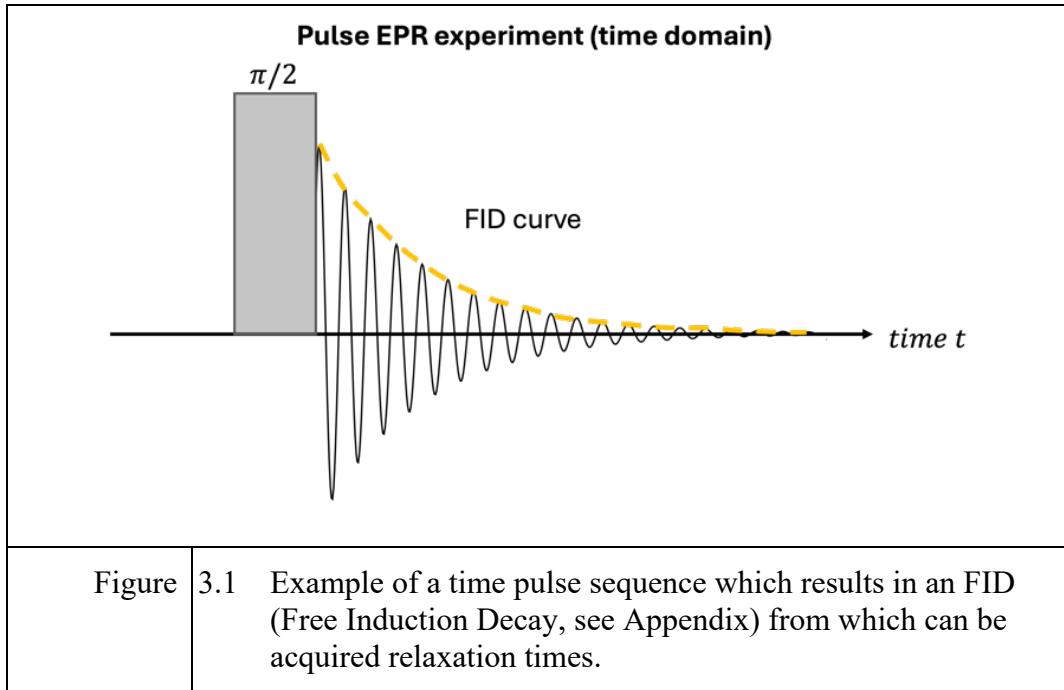
Figure	3.1	Example of a time pulse sequence which results in an FID (Free Induction Decay, see Appendix) from which can be acquired relaxation times.

Amplitude modulation in EPR means a technique whereby the static magnetic field is varied-that is, modulated periodically through a small alternating magnetic field of fixed frequency, usually in the kHz range. This modulation makes the EPR signal oscillate at the modulation frequency; it can then readily be isolated and amplified by using phase-sensitive detection. It results in an improved signal-to-noise ratio and thus records the derivative of the absorption spectrum. The technique is quite important for enhancing sensitivity and rejecting background noise to levels that permit the detection of weak paramagnetic signals. It is important to choose the correct modulation frequency range to avoid overmodulated spectra, which can lose important information about the measured sample. [modulation]

The line shape in EPR is the detailed profile of a resonance signal, that might carry information on basic physical and chemical processes to which the spin is subjected. The most common forms include the *Lorentzian* associated with a homogeneous broadening by uniform spin interactions, and the *Gaussian* associated with an inhomogeneous broadening due to variations of the local magnetic environment. [line width line shape]

Linewidth usually refers to the full width at half maximum (FWHM) and depends on several factors that include
- Spin-spin relaxation (T_2)
    - Shorter T_2 times lead to broader lines due to faster loss of coherence between spins.

- Hyperfine interactions
  - o Coupling between unpaired electrons and nearby nuclei can split or broaden the signal.

- Magnetic field inhomogeneities
  - o Variations in the field across the sample can cause additional broadening.

- Modulation
  - o Over modulation can broaden the spectra hence loos the valuable information.

[here] to je BOLTON
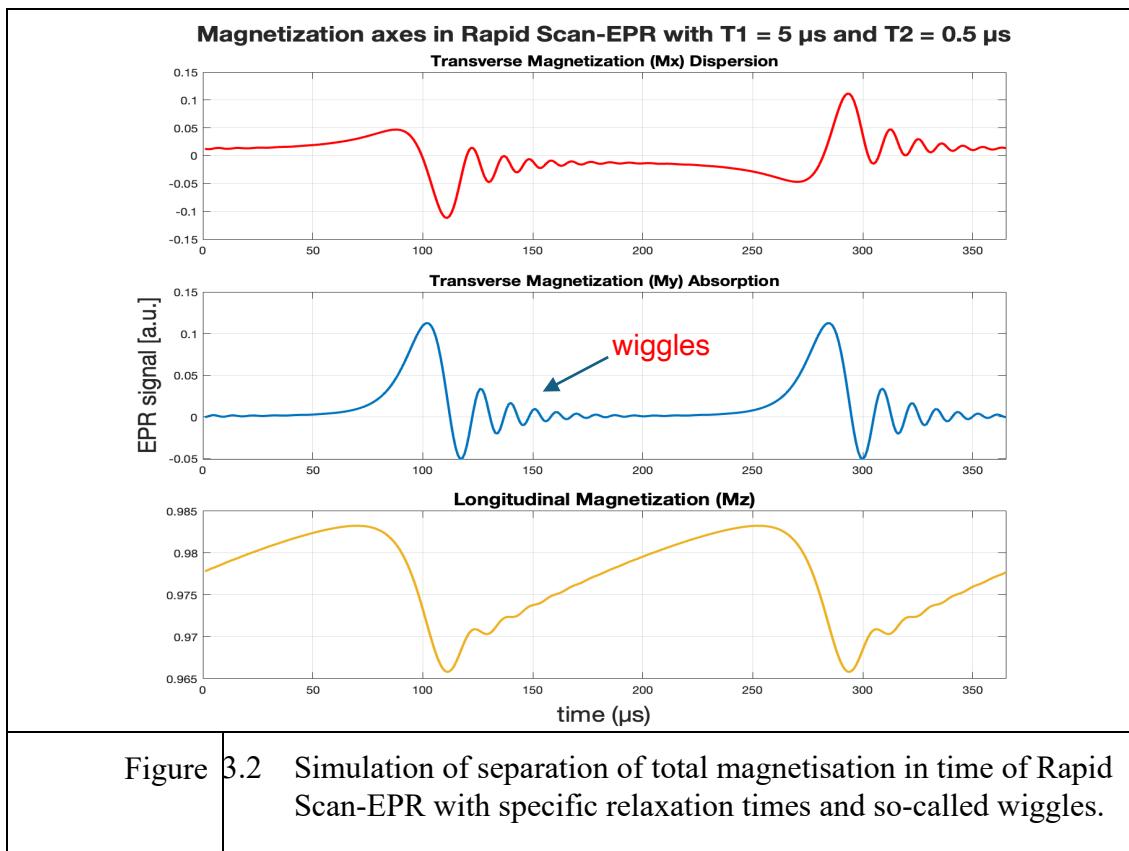
3.2 RAPID SCAN – EPR

An alternative to earlier mentioned methods is the rising in popularity method called Rapid Scan EPR. It is a between method of CW and Pulse. Rapid Scan can provide detailed analysis possible for spin relaxation times, especially that of the transverse relaxation time T_2. This is performed through the rapid sweeping of the microwave frequency or magnetic field. In addition, high-power microwave pulses or specially developed resonant cavities are not required in this method, as is the case in some traditional pulse ESR techniques. This method has been developed in a cost-effective manner and can be versatile for many experimental configurations. BOLTON, EATON,

Its principle is based on a rapid modulation of the excitation parameters-for example, the microwave frequency or the magnetic field-inducing specific distortions in the EPR spectrum. If the sweep rate is high enough, transient oscillations, called "wiggles", show up in the spectrum and may contain information about the dynamics of the spin system, such as the T_2 relaxation time (see Figure 3.2). This is advantageous in that the method avoids the dead time limitations of conventional pulse EPR and can access T_2 values on the nanosecond scale. The relationship between the relaxation time T_2 and the required sweep rate $\frac{d\omega}{dt}$ to observe these oscillations is given by: [laguta]

$$\left|\frac{d\omega}{dt}\right| \geq \frac{1}{T_2^2} \qquad\qquad (\text{eq. } 3.1)$$

Where ω represents the microwave frequency. This condition highlights the importance of achieving extremely high sweep rates for systems with short T_2 times.

Figure 3.2   Simulation of separation of total magnetisation in time of Rapid Scan-EPR with specific relaxation times and so-called wiggles.

In the rapid scan ESR, microwave frequency sweep is usually preferred over magnetic field sweeps due to much faster modulation rates offered by this approach. Frequency sweeping in the terahertz range is feasible in modern microwave sources, while magnetic field sweeping is usually restricted by technical limitations. The frequency domain implementation allows the Rapid Scan EPR to cover extensive spectral ranges within one measurement, hence offering all-round information about the spin system. [laguta]

Oscillations that appear in spectra recorded with rapid cans are an interplay between the sweep rate and the spin-relaxation dynamics. Such oscillations feature properties like those of a damped FID signal, where its periods progressively decrease. Thus, these signals must be analysed by deconvolution techniques and numerical fitting to obtain the values of T_2. The shape of the spectrum is a function of the form of the sweep profile $\omega(t)$ and, typically features linear or sinusoidal, of the investigated spin system. This analysis can be further improved using modified Bloch equations considering the effects of rapid sweeps. These equations represent a firm theoretical basis on which the simulation and interpretation of the experimental data have been performed, especially for systems presenting inhomogeneous line broadening due to g-factor anisotropy, unresolved hyperfine interactions, or sample inhomogeneity.

[LAGUTA]

3.2.1 Modified Bloch equations

This brings us to the analysis and interpretation of data from Rapid Scan EPR experiments, necessitating the modification of the so-called Bloch equations describing the dynamics of magnetisation under the action of relaxation and excitation fields. Modifications allow for such conditions as rapid frequency or magnetic field sweeps, which can precisely model transient oscillations ("wiggles") observed in the spectra of a rapid scan.

In the context of Rapid Scan EPR, the magnetization components M_x, M_y and M_z are expressed in a rotating frame of reference. The modified Bloch equations are written as:

$$\frac{dM_x}{dt} = -\frac{M_x}{T_2} - [\Delta\Omega + \omega(t)]M_y \qquad \text{(eq. 3.2)}$$

$$\frac{dM_y}{dt} = [\Delta\Omega + \omega(t)]M_x - \frac{M_y}{T_2} - \gamma B_1 M_z \qquad \text{(eq. 3.3)}$$

$$\frac{dM_z}{dt} = \frac{M_0}{T_1} + \gamma B_1 M_y - \frac{M_z}{T_1} \qquad \text{(eq. 3.4)}$$

where M_x, M_y and M_z are projections of the total magnetization M_0, $\Delta\Omega$ is the offset of the resonance line from the center of the sweep $\omega(t)$, and B_1 is the magnitude of the magnetic component of the microwaves. The numerical solution of these equations followed by fitting of the measured spectrum allows extraction of T_2 (and also T_1) time. [laguta]

4. DNP

Dynamic Nuclear Polarization (DNP) enhances NMR sensitivity by transferring polarization from electron spins of radicals to nuclear spins via microwave irradiation. This is largely driven by the Overhauser effect in the liquid state (not only liquid state but this state is chosen for this work). The process depends on factors like coupling, saturation, and leakage, which all depend on the relaxation times of polarizing agents. Determinations of electron T_1 and nuclear T_1 are of paramount importance in optimizing the efficiency of DNP by accurately controlling polarization transfer and stability of the signal. Only with rapid-scan EPR would proper characterization of radicals be possible, thereby informing on spin dynamics, exchange interactions, and relaxation properties. This combination of the measurements of relaxation and EPR analysis does ensure effective design of polarizing agents that can greatly maximize DNP performance at high-field NMR conditions, especially those pertaining to biological conditions. [neugeabuer 2013 a 2014]

4.1 Basics of DNP mechanisms

A range of fundamental mechanisms determines the efficiency of DNP, describing the interaction of electron and nuclear spins for polarization transfer. These mechanisms essentially depend on the physical state of the system, whether a solid, liquid, or gas, properties of the polarizing agent, and, finally, experimental conditions, that is, magnetic field strength and temperature.

4.1.1 Polarization Transfer Mechanisms

There are four primary mechanisms through which DNP operates, each associated with specific spin dynamics:
1. Overhausser effect (dominant in liquids) [https://doi.org/10.1021/ja00171a049]
    The Overhauser effect comprises dynamic couplings of electron and nuclear spins by both scalar and dipolar couplings. Irradiation at microwave frequencies on resonance for the electrons provokes transitions in the state of the electron spins, which, via the relaxation processes, affect nuclear spin polarization. This mechanism is very sensitive to molecular motion and finds its most effective conditions in liquids where fast molecular tumbling occurs.

2. Solid effect [https://doi.org/10.1039/C6CP04621E]
    The solid effect can take place in those systems only where strong dipolar interactions between electrons and nuclei exist. It involves the simultaneous microwave-driven transitions of a flip of the electron spin and a flip of nuclear

spin. This is an important mechanism in solids, but it requires high microwave power and a narrow EPR linewidth.

3. Cross effect [https://doi.org/10.1039/C6CP04621E]
   This involves a cross effect between two unpaired electron spins and a nuclear spin. The main condition of this is when the difference in electron resonance frequencies matches the nuclear Larmor frequency. This is an effective mechanism in high-field DNP and often exploited using biradicals as polarizing agents.

There are also other effect which are used in the DNP methods, but the Overhausser effect and Cross effect are used for this kind of work.

4.1.2 Key factors influencing DNP

Several factors determine the efficiency and outcome of DNP experiments:

1. Relaxation Times:
   I. Electron Relaxation (T_1e and T_2e):
      These govern the rate at which electron spins return to thermal equilibrium and how well they respond to microwave saturation.

   II. Nuclear Relaxation (T_1):
      Determines the longevity of enhanced nuclear polarization.

2. Microwave Power:
   Adequate microwave power is needed to saturate electron spins, maximizing the polarization transfer rate.

3. Coupling Factor (ξ): [https://doi.org/10.1016/j.jmr.2020.106719]
   Describes the efficiency of electron-nuclear interactions, influenced by the type of coupling (scalar or dipolar) and molecular dynamics.

4. Polarizing Agents:
   Radicals with optimized spin properties (e.g., short T_1e and narrow linewidths) are crucial for achieving high DNP efficiency.

5. Environmental Factors:
   Solvent viscosity, temperature, and the magnetic field strength directly affect molecular motion and relaxation dynamics, influencing the dominant DNP mechanism.

4.2 Polarizing agents

Polarizing agents are paramagnetic media, most often free radicals, which provide the unpaired electron spins required for polarization transfer to nuclear spins in DNP. Design considerations for polarizing agents seek increased polarization efficiency with stability of the agent under the conditions of the experiment. Polarizing agents operating in the liquid phase have to have properties that enable the Overhauser effect-important DNP mechanism in this phase.

4.2.1 Properties of polarizing agents

There many specifics from to choose to describe the polarization agents by some of its properties, but, in this subsection we are mainly are going to focus on two major ones. These properties are:

1. Electron Spin Density [10.1039/D3SC01079A]
   A large spin density thus ensures strong dipolar or scalar interactions between the unpaired electron spins and nuclear spins for efficient polarization transfer.

2. Relaxation Times
   T_1-Longitudinal relaxation:
   > Short T_1 ensures rapid transfer of polarization from electrons to lattice (nuclei in the case of DNP).

   T_2-Transverse relaxation:
   > Long T_2 effectively supports microwave saturation, which is necessary to retain the electron spin in a non-equilibrium state during DNP.
   > Such knowledge of these relaxation times is very important for performance optimisation of polarising agents and hence yielding efficient Overhauser effect dynamics.

   In DNP, the relaxation times of polarizing agents directly impact the efficiency. During the process of liquid-state DNP, a very short T_{1e} ensures that electron spins will more rapidly return to thermal equilibrium for continuous polarization transfer. Long T_1 restores the polarized state for subsequent NMR detection. The inequality between these two relaxation times may reduce the overall enhancement from DNP; thus, proper characterization of polarizing agents becomes vital to maximize experiments.
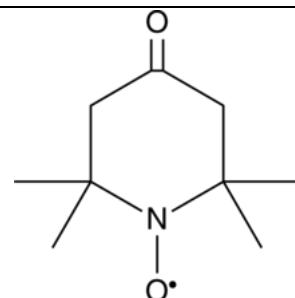
4.2.2 Common Polarizing Agents

Some of the most used types of liquid polarizing agents are nitroxide radicals, trityl radicals, and biradicals.

1. Nitroxide radicals: [10.1039/C8CP05236K] [acetone_tempo]
   Nitroxide radicals, such as TEMPOL and TEMPONE, are polarizing agents commonly employed in liquid-state DNP due to their chemical stability, water solubility, and efficient polarization transfer capabilities. Such radicals have high electron spin density, which enables strong coupling between the electron and nuclear spins, thus easily transferring the polarization from electron spins to nuclear spins through the Overhauser effect. Their relaxation properties also tend to be benign, with very short T_1e and relatively long T_2e, thus efficiently polarizing while maintaining signal enhancements. Among nitroxide radicals, good solubility, stability, high DNP efficiency, and appropriate line-shape characteristics make them the most promising radicals in biological and aqueous systems. Proper measurement of their relaxation times is necessary for the optimal conduct of DNP with these radicals, a way to optimize the experimental parameters toward efficient NMR signal enhancement.

DESIGN OF THE DATABASE

EXPERIMENT PREPARATION

SAMPLE PREPARATION